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New look at the etiology of chronic pyelonephritis among children with polymorphism of TLR2 gene

Keywords: polymorphism, Toll-like receptors, bacterial infection, intracellular pathogens.

Nephrological pathology is one of the most widespread and at the same one the most difficult to treat in the pediatric practice. This is due to the peculiarities of the urinary system, its high vulnerability to infectious diseases, their tendency to chronicity and limitations to the use of some medications. Although many factors leading to the development of pyelonephritis (PN) known (obstruction, dismetabolic violations, heredity, etc.), however, in 41% of children sclerosis develops in the absence of urodynamic disorders, that is the direct cause of renal damage among children is an infection that accompanied by local activation and launching cytotoxic metabolism. This category of children deserves special attention, because the frequent infections can lead to basic disruption of compensatory-adaptive mechanisms lead to significant disturbances of the functional state of the body, which reduces immune resistance and lead to early development of chronic diseases [1, 2, 3].

During last year's, thanks to the progress of scientific disciplines such as molecular biology, genetics, immunology and cell biology, the accumulation of knowledge about the mechanisms of various infectious diseases, and it becomes possible to accurate diagnosis and prognosis. We know that the state of resistance to infection is formed by numerous reactions of the immune system, which main function is the recognition and elimination of infectious agents and their metabolic products [4, 5, 6, 7, 8].

Recently more and more confirmation gets the hypothesis of K. Janeway about exceptional importance of innate immunity in the protection from pathogens and in the initial stages of adaptive immune reactions. The cells of innate immunity distinguish the conservative in evolutionary terms molecules typical to both large taxonomic groups of microorganisms. Toll-like receptors (TLR) have key role among the distinctive receptors of innate immunity. After interaction with the TLR ligand there is an activation signal pathways, which are produced as a result of effectors molecules such as cytokines, antimicrobial peptides, etc. [12, 13, 17]. In coordinated operation of innate immune factors in most cases is elimination of pathogens. Defects in the Toll-like receptors system, such as violations of recognition ligands, expression of TLR, signal transduction, synthesis of effectors molecules and gene polymorphism Toll-like receptors can lead to the development of severe infectious diseases, autoimmune diseases, atherosclerosis, allergic pathology etc. [14, 15,16].

Our attention was caught to polymorphisms TLR4 Asp299Gly, because there are some research that indicate that the presences of these mutant alleles of genes that increase the risk of infection of urogenital infections such as chlamydiosis, mycoplasmosis, ureoplasmosis, hardnelosis, trichomoniasis [8,9].

Polymorphisms of genes suggests that the same gene may be copied several different structural copies of the same protein, with some variations copied or do not active has or may have the opposite function. As the differences in the genes which control the body's protective response may determine the different nature of the inflammatory response and specific immunological reactions when introducing of foreign agents, the research of the children prevalence single nucleotide substitutions with chronic pyelonephritis (PN) is of our particular interest [10,11,15,16].

The aim of our research was to assess the current position of importance of polymorphism Asp299Gly Toll-like receptor 4 in the implementation of susceptibility to chronic pyelonephritis in childhood and to analyze its association with major pathogens of the urinary system infections.

Objects and methods of research. A clinical and laboratory examination of 53 children with chronic pyelonephritis during exacerbation who were hospitalized in the pediatric department number 2 (Renal beds) Children's Regional Hospital Poltava was conducted.

Selection of biological samples from patients, which was observed, was conducted in the absence of infectious diseases. The material for the research was peripheral venous blood sampling which was conducted cube vein in sterile container with stabilizer (DTA), followed by making it into ependorfy of reagent "DNA-Express". Genotyping polymorphic sites Asp299Gly performed by polymerase chain reaction (PCR) using oligonucleotide primers. Amplification was carried out on Thermocyclers "Tertsyk" ("DNA Technology", Moscow).

Bacteriological examination of urine with the defining microbial pathogen and its susceptibility to antibiotics and chemotherapeutic agents and molecular biological researches (polymerase chain reaction to determine Chlamidia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis, material research - epitelialnyk scraping cells from the urethra) was performed in the majority of patients to antibiotic therapy.

The control group consisted of 95 healthy individuals with genetic base of samples Institute of Genetic and immune basics of pathology and pharmacogenetics Poltava National Medical And Dental University "UMSA." From all patients received voluntary written consent to participate in research, which was conducted with the permission of the Commission on Bioethics State Higher School of Ukraine "Ukrainian Medical Dental Academy".

Mathematical treatment of the data was performed using the program «STATISTICA for Windows 7.0» (StatSoft Inc) and spreadsheets MS Excel. The distribution of genotypes in the investigated polymorphic loci was tested for compliance with Hardy-Weinberg equilibrium using the criterion $\chi 2$. To compare allele frequencies between the investigated groups, using Pearson's $\chi 2$ test was corrected for continuity Yets when the number of degrees of freedom, which is equal to 1. Comparison of genotype frequencies between investigated groups was performed by analysis of interface tables using Fisher's exact test. For comparison, the frequency of choices in unrelated groups was calculated the odds ratio (OR) with 95% trusting defined interval (CI). Statistically significant for all types of analysis was considered differences at $p < 0.05$ at $0,05 \square p \leq 0,1$ noted a trend towards differences.

Results.

The analysis of the frequency distribution of genotypes in the investigated TLR2 Arg753Gln gene in monitored groups is provided in Table 1. As can be seen from the table, "wild-type" genotype of TLR2 of healthy persons of Poltava region met in 97.9%. While as among children with chronic pyelonephritis frequency of heterozygous GA genotype more than 4 times often the indicators of the control group (9.4 and 2.1 respectively) ($p < 0,05$). Homozygous AA genotype was not detected, either in healthy persons or patients with chronic pyelonephritis.

Table 1. The distribution of genotypes and allele frequencies of polymorphism TLR2 Arg753Gln gene among healthy individuals and children with chronic, % (n)

Gene, polymorphism	The frequency of genotype, allele	Control group, n=95	Children with chronic pyelonephritis, n=53
TLR2 Arg 753Gln	GG	97,9% (93)	90,6% (48)
	GA	2,1% (2)	9,4% (5) *
	AA	0%(0)	0% (0)
	G	98,9% (188)	95,2% (101)
	A	1,1% (2)	4,8% (5)*

** p <0.05 compared with the group of healthy persons of Poltava region*

The research of the prevalence of normal and mutant alleles of the gene 753Gln Toll-like receptor 2 in tested groups showed that the mutant allele Arg met 4 times often (4.8%) in group of children with chronic pyelonephritis compared with the group of healthy persons of Poltava region (1.1%) ($p < 0,05$).

Genetic markers may determine tendency to disease in general, or to be associated with specific etiopathogenetical significant features. Therefore, as part of our work is an important step was the analysis of the association between the polymorphism of TLR2 gene 2 (Arg753Gln) of the main pathogens of the urinary system among children with chronic pyelonephritis (Table 2). To solve this problem, we formed two subgroups of patients with chronic pyelonephritis children: first – children heterozygous for the mutant allele genotype (Gln/Arg, n=5) and children with normal distribution of alleles that are included in the comparison group (n=45).

The results of molecular biological examination among children with chronic pyelonephritis indicate that the frequency of detection of mycoplasma infections in infants subgroups almost 4 times higher the indicators of comparison group (40% and 11.1% respectively). Ureaplazma ureolyticum also 2 times more often among children with recorded mutant allele genotype versus among children with normal distribution of alleles (20% and 11.1% respectively). Intracellular pathogens in the vast majority of children in both subgroups revealed as monoinfection (60% and 93.53%, respectively), and the definition of mixed infection

was recorded significantly higher in the subgroup of infants versus children with normal distribution of alleles (40% and 2.2% respectively).

The results of bacterial examination of patients with chronic pyelonephritis indicate that all children first subgroup was detected E. coli, in contrast to the comparison group, where it was determined only in 15.6% of children. It should be noted that children with distribution of mutant alleles was the only bacteriological agent, in contrast to the control group, where in the vast majority of children (80%) did not determine the bacterial flora in the small and almost equivalent to the percentage recorded enterobacteria, klebsiyella and Proteus.

Table 2. Association of genotypes analysis with the major etiological agents of chronic pyelonephritis of genetically examined children n (%)

Etiological factor	Children with GA genotype		Children with GG genotype	
	absolute	relative, %	absolute	relative, %
Chlamidia trachomatis	0	0	5	11,1
Ureaplazma ureolyticum	1	20*	5	11,1
Mycoplazma hominis	2	40*	5	11,1
Intracellular pathogens were found	2	40	32	71,1
Identified associations intracellular pathogens	0	0	2	4,4
Escherichia coli	5	100*	7	15,6
Proteus	0	0	1	2,2
Klebsiella	0	0	1	2,2
Enterobacteria	0	0	2	4,4
Microbial Association	0	0	2	4,4
Mixed infection	2	40*	1	2,2
Flora is not allocated	0	0	36	80

** $p < 0.05$ compared with the second group*

Conclusions: Thus, the data indicate that heterozygous carriers of the mutant allele can be considered as a predictor of increased risk of chronic pyelonephritis, which is characterized by the formation of phenotypic manifestations of the disease, with a prevalence of recurrent and torpid course of inflammatory changes, and the need for long-term maintenance uroseptic therapy. After all for today found that 50-65% of children with inflammation leads to irreversible damage to renal parenchyma with substitution of damaged sections of connective tissue, which subsequently leads to the development of chronic renal failure (CRF) and hypertension [1, 2, 6]

After examining of genotypes features of chronic pyelonephritis children with polymorphisms of the gene Toll-like receptor 2 is determined that heterozygotes for the mutant allele genotype is associated with high sensitivity to Escherichia coli and increased sensitivity to key intracellular pathogens (Mycoplazma hominis, Ureaplazma ureolyticum) pyelonephritis.

These results can be explained by the fact that TLR2 recognizes a wide variety of ligands pathogens and includes mechanisms for their elimination from the body. It can be assumed that substitution Gln for Arg at position 753 results in a conformational change in the TIR-domain and signal violations of TLR2, accompanied by an imbalance of the production of antimicrobial peptides and cytokines, resulting in an active infection. Therefore, the definition of polymorphism of the gene Toll-like receptor 2 among children with chronic pyelonephritis can be considered as an additional prognostic marker of recurrent disease with a tendency to stable bacteriauri. After all, an individual approach to the children with chronic pyelonephritis, based on the scientific interpretation of the results of genetic research comparing them with the data of clinical, laboratory and instrumental methods, allows for early diagnosis of genetically determined diseases and offer the most effective scheme of preventive and therapeutic measures for preventing the progression of pathological process and resultant patient morbidity.

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Summary. Currently the mechanisms of recognition of alien agents, which is implemented by Toll-like receptor innate immune system has become one of the main tasks of clinical immunology. The aim of our research was to analyze the association between polymorphism of the gene Toll-like receptor 2 (Arg753Gln) of the main pathogens of the urinary system. These results confirm the important role of Toll-like receptors in the realization of innate immune and allow considering polymorphism Toll-like receptor gene 2 as an additional prognostic indicator in genetic researches.

Key words: innate immunity, polymorphism, Toll-like receptors, bacterial infection, intracellular pathogens.